ULTRASONOGRAPHY OF THE UROGENITAL ORGANS OF MALE WATER MONITOR LIZARD (*Varanus marmoratus,* Weigmann, 1834)

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ABSTRACT

Eight apparently healthy male water monitor lizards *(Varanus marmoratus*), weighing 0.5-4.8 kg, were examined using an ultrasound machine equipped with a 5.0 MHz linear array scanner to determine the ultrasound appearance, dimensions and echo mean values of the urogenital organs. Based on snout to vent length, the eight animals were equally divided into small (<70 cm) and large (>70 cm) varanids. The kidneys of varanids were observed caudal to each gonad. They were elongated with hypoechoic parenchyma and hypoechoic to hyperechoic interlobular spaces. The testes of all eight males appeared as homogenous and moderately echogenic structures. The hemipenes appeared as anechoic structures with hypoechoic walls. The urinary bladder appeared as an elongated anechoic structure with hypoechoic walls. Significant differences in the length of left and right kidneys, length, width and circumference of left testes, length of hemipenes and length of the urinary bladder across sizes were observed. No differences were observed in the echo mean values of the different organs among small and large varanids. The data obtained in the study could serve as baseline values for evaluation of diseases and disorders of the male urogenital organs in water monitor lizards.

Keywords: hemipenis, kidney, testes, urinary bladder, Varanus, water monitor lizard

INTRODUCTION

Water monitor lizard *(Varanus marmoratus,* Weigmann, 1834) is classified under the Kingdom Animalia, Phylum Chordata, Class Reptilia, Order Squamata, Family Varanidae and Genus Varanus (Gaulke, 1992). The term “varanus” came from the Arabic word “waral” or “waran” meaning “lizard”. The water monitor lizard or *bayawak* (Tagalog) can grow up to 2.1 m in length, reaching weights up to 25 kg. The habit of varanids to stand on their hind legs and appear to be monitoring led to its name “Monere”, a Latin word meaning “to warn”. Philippine monitor lizards include six species: water monitors (*Varanus cumingi, Varanus marmoratus* and *Varanus nuchalis*), and arboreal monitors (*Varanus olivaceus, Varanus bitatawa* and *Varanus mabitang*) (Welton *et al.*, 2010).

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In the wild or in captivity, varanids may succumb to deficiencies and diseases that may lead to their death. The challenge for conservationists and veterinarians is to formulate a diagnostic protocol which will provide an accurate and quick diagnosis to be able to provide proper treatment. Among the various means of diagnosis, ultrasonography or diagnostic ultrasound has been proven to be a popular non-invasive tool for characterizing lesions in different organs.

Ultrasonography has been utilized to explore soft tissues in reptiles including the liver, kidney, bladder, genital system, heart and digestive tract (Sainsbury and Gilic, 1991; Isaza *et al.*, 1993; Schildger *et al.*, 1994; Hernandez-Divers and Hernandez-Divers, 2001; Kik, 2002; Hochleitner and Hochleitner, 2004; Valente, 2007; Enriquez *et al*., 2011; Aguisanda *et al.*, 2011). In contrast with radiography, it can differentiate various soft tissue structures and provide information regarding organ location and pathologic changes (Hernandez-Divers and Hernandez-Divers, 2001). However, although ultrasonography has been utilized in snakes (Isaza *et al.*, 1993; Snyder *et al.*, 1999, Schilliger *et al.*, 2005; Enriquez *et al*., 2011; Aguisanda *et al.*, 2011), iguana (Smith *et al.*, 2001), tortoise (Penninck *et al.*, 1991) gila monsters and beaded lizards (Morris and Alberts, 1996), limited studies have been conducted in water monitor lizards. Ultrasonography (100%) has been observed to be more accurate than contrast radiography (81.4%) in determining the gender of snakes (Gnudi *et al.*, 2009).

At present, there are no known published studies conducted in water monitor lizards (*Varanus marmoratus*). Ultrasonography can help properly determine sex, diagnose internal abnormalities and diseases and evaluate pregnancy. Baseline data on the ultrasonographic features of the urogenital system can help improve current captive management and breeding practices, enabling the clinician to provide proper care and treatment for the animals. This study was conducted to determine the ultrasonographic features of the urogenital tract of male water monitor lizards raised in captivity.

MATERIALS AND METHODS

Eight apparently healthy male water monitor lizards (*Varanus marmoratus*), weighing 0.5-4.8 kg, with snout to vent length of 56-87 cm and total length of 92-137 cm, were utilized in the study. Based on snout to vent length, the eight animals were divided into small (<70 cm, 4 animals) and large (>70 cm, 4 animals) varanids. Measurement of snout to vent length has been shown to aid in determining approximate age of the animal. According to Bennett (1998), sexual maturity of female and male varanids is attained when it has snout to vent length of around 120 cm and 130 cm, respectively.

Physical examination was conducted prior to the experiment to ensure that the animals were apparently healthy. Only apparently healthy animals based on history and physical examinations were included in the study. Animals with signs of lethargy, presence of nasal discharges, tumors, wounds and deformities were excluded in the study.

The water monitor lizards were obtained from the Protected Areas and

Wildlife Bureau, Wildlife Rescue Center (PAWB-WRC), Department of Environment and Natural Resources, Diliman, Quezon City. The identification, age, diet and date of acquisition of the lizards were obtained from the records of the institution. The species were identified using identification keys based on descriptions by Koch *et al.* (2010).

The animals were properly restrained manually in accordance to the techniques described by Barten (2006), and placed in dorsal recumbency. Ultrasonic gel was applied to the transducer and the skin to improve contact. The varanids were scanned using an ultrasound machine (Aloka Ultrasound Diagnostic Equipment, Aloka® SSD-500, Aloka Co. Ltd., Tokyo, Japan) equipped with a 5.0 MHz linear array scanner. Machine settings of overall gain: 70 dB, near gain: 15.0 dB and far gain: 2.5 dB, were maintained for all animals. The scanner was placed on the abdomen and directed cranial to caudal and medial to lateral, to visualize the different urinary and reproductive organs. The ultrasonograms obtained were printed out and used to determine the size and shape of the organs. Length, width and thickness of the organs were measured. In addition, the echogenicity of the organs was determined through histogram analysis using Adobe Photoshop 6.0 (Adobe Systems Incorporated, 1998-2000) and was expressed as echo mean value.

All data collected were subjected to Student's t-test to determine the presence of significant variation in all variables between sizes: small and large at $\alpha = 0.05$. The values obtained from all parameters were tested for normality using Wilk-Shapiro test.

The protocol for this study has been approved by the Protected Areas and Wildlife Bureau of the Department of Environment and Natural Resources and the Institutional Animal Care and Use Committee of the University of the Philippines Los Baños College of Veterinary Medicine.

RESULTS AND DISCUSSION

The kidneys, urinary bladder, testes and hemipenes were visualized using diagnostic ultrasound in all animals. In a few animals, there was a slight difficulty in obtaining clear sonogram images due to the presence of highly keratinized scales of the varanids. This was remedied by placing plenty of ultrasound gel in the scales of the varanids before scanning. Barriers in ultrasound examination of reptiles which limit penetration of sound waves and create artifacts have been recognized. These include keratin on the reptile scales, the presence of bones (ribs, girdles and dermal bones) and air (lung air and intestinal gas because of the lack of a diaphragm) and the interference of the unshed dermis and the underlying air during ecdysis in snakes and lizards (Sainsbury and Gilic, 1991; Isaza *et al.*, 1993; Schildger *et al.*, 1994; Hochleitner and Hochleitner M, 2004). To minimize these artifacts, copious amounts of acoustic coupling gel can be liberally applied on the skin, at least 5 min before application of the transducer to reduce trapped air on the irregular skin and under the scales. Standoff pad utilizing a latex glove filled with gel placed between the skin and the transducer allows for better image quality. Finally, the patient can be partially submerged in a warm water bath (Stahl, 2007) so that transducer-skin contact will not be necessary. This can facilitate imaging especially in areas of the skin with convex surfaces. The probe can even be pressed with gel against the plastic container itself (Sainsbury and Gilic, 1991; Isaza *et al.*, 1993; Schildger *et al.*, 1994; Hochleitner and Hochleitner M, 2004).

The organs from large varanids were more easily recognized than those in small varanids, in agreement with the findings of Enriquez *et al.* (2011) in pythons. Stahl (2007) recommended the use of higher frequency transducers with good standoff pad if it is inevitable to utilize juvenile lizards. For reptile examination, different frequencies have been recommended. Smaller animals (snakes, most lizards and small chelonians) require the use of a 10-15 MHz, medium to large size patients require 5-8 MHz transducer while a 3.5 MHz scanner is warranted for very large reptiles such as big crocodiles, giant tortoises, large monitor lizards or sea turtles (Hernandez-Divers and Hernandez-Divers, 2001; Silverman, 2006; Stetter, 2006). In this study, however, it was observed that 5.0 MHz frequency can provide good quality images for scanning of water monitor lizards.

The urogenital organs in the male monitor lizard were visualized when the animal was placed in dorsal recumbency and the scanner was positioned on the ventral surface of the animal. This agrees with the recommended acoustic window for reptiles, except for chelonians (Schildger *et al.*, 1994; Hernandez-Divers and Hernandez-Divers, 2001; Hochleitner and Hochleitner M, 2004; Silverman, 2006; Stetter, 2006; Enriquez *et al*., 2011).

Kidneys

The kidneys in all animals were visualized in the lateral aspect of the caudal abdomen, caudal to the gonads, beneath the intra-abdominal adipose bodies and within the pelvic area of the animal. This agrees with the location of the kidneys described in other reptiles (Sainsbury and Gilic, 1991; Isaza *et al.*, 1993; Schildger *et al.*, 1994; Hernandez-Divers and Hernandez-Divers, 2001; Hochleitner and Hochleitner, 2004). In imaging the kidneys of reptiles, the transducer can be placed against the sublumbar fossa and directed towards the pelvic inlet; it can also be placed against the lateral base of the tail and the beam directed cranially toward the pelvis outlet. In snakes, the right kidney is cranial in relation to the left kidney (Sainsbury and Gilic, 1991; Isaza *et al.*, 1993; Schildger *et al.*, 1994; Hernandez-Divers and Hernandez-Divers, 2001; Hochleitner and Hochleitner, 2004).

The kidneys of the varanids were not divided into medulla, cortex and pelvis as in the case with mammals (Nyland and Mattoon, 1995; Kealy and McAllister, 2000; Mannion, 2006), in agreement with the findings of Sainsbury and Gilic (1991), Isaza *et al.* (1993), Schildger *et al.* (1994), Hernandez-Divers and Hernandez-Divers (2001), Hochleitner and Hochleitner (2004) and Stahl (2007). The sonograms showed an elongated organ with no distinction between medulla and cortex (Figure 1). The kidneys appeared homogenous with hyperechoic and hypoechoic transverse partitions, which represent the interlobular spaces. Compared to the surrounding intra-abdominal adipose bodies, the kidneys appeared more hyperechoic and granulated, in agreement with previous observations (Frye, 1991; Sainsbury and Gilic, 1991; Isaza *et al.*, 1993; Schildger *et al.*, 1994; Hernandez-Divers and Hernandez-Divers, 2001; Hochleitner and Hochleitner, 2004; Stahl, 2007).

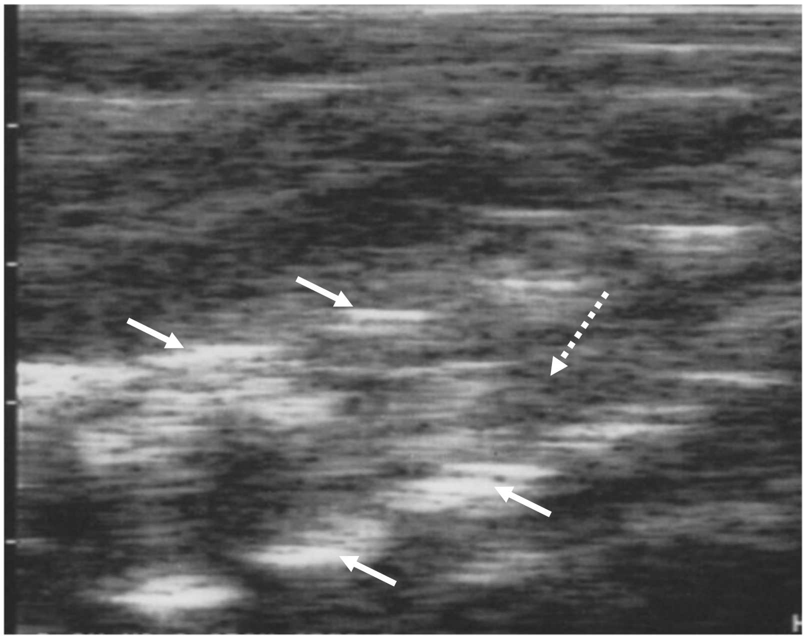

Figure 1. Longitudinal ultrasound scan of the kidney in water monitor lizard (*Varanus marmoratus*) showing hyperechoic (solid arrows) to hypoechoic walls and hypoechoic parenchyma (dotted arrow).

The dimensions and echo mean values of the left and right kidneys are shown in Table 1. The results showed no significant differences on the mean width of kidneys between small and large varanids. However, significant differences on length were observed between sizes. Bigger animals have longer left and right kidneys than smaller specimens. Histogram analysis revealed no significant differences in the echo mean values of the left and right kidneys between sizes. This agrees with the findings of Acorda *et al.* (2009) who found no differences in the echogenicity of the kidneys in sheep at different ages.

Urinary bladder

The urinary bladder was visualized in the ventral midline, slightly cranial to the pelvis. Mader (2006) reported that the urinary bladder occupies the caudal coelom while other studies stated that the urinary bladder is located in a caudoventral position, cranial to the pelvis (Sainsbury and Gilic, 1991; ; Isaza *et al.*, 1993; Schildger *et al.*, 1994; Hernandez-Divers and Hernandez-Divers, 2001; Hochleitner and Hochleitner, 2004).

The urinary bladder appeared in the ultrasonogram as a thin-walled anechoic structure with hypoechoic walls (Figure 2), similar to the descriptions in other studies (Frye, 1991; Sainsbury and Gilic, 1991; Isaza *et al.*, 1993;

Table 1. Mean±S.D. dimensions and echo mean values of the kidneys and urinary bladder of *Varanus marmoratus* (Weigmann, 1834).

Organ / Animal	Length (cm)	Width (cm)	Echo mean value
Left kidney			
Small	8.19±0.19[a]	2.32±0.60[a]	90.51±6.56[a]
Large	8.69±0.10[b]	2.40±0.39[a]	90.55±6.55[a]
Average	8.44±0.32	2.36±0.4	90.53±6.56
Right kidney			
Small	8.22±0.12[a]	2.56±0.33[a]	89.19±6.18[a]
Large	8.80±0.20[b]	2.58±0.69[a]	91.45±5.11[a]
Average	8.51±0.38	2.57±0.49	90.32±5.73
Urinary bladder			
Small	3.86±0.73[a]	1.13±0.46[a]	47.92±6.08[a]
Large	4.40 ±1.12[b]	1.19±0.53[a]	47.94±8.70[a]
Average	4.13±0.84	1.16±0.55	47.93±6.42

Means in the same column from the same organ with different superscripts are different (P<0.05).

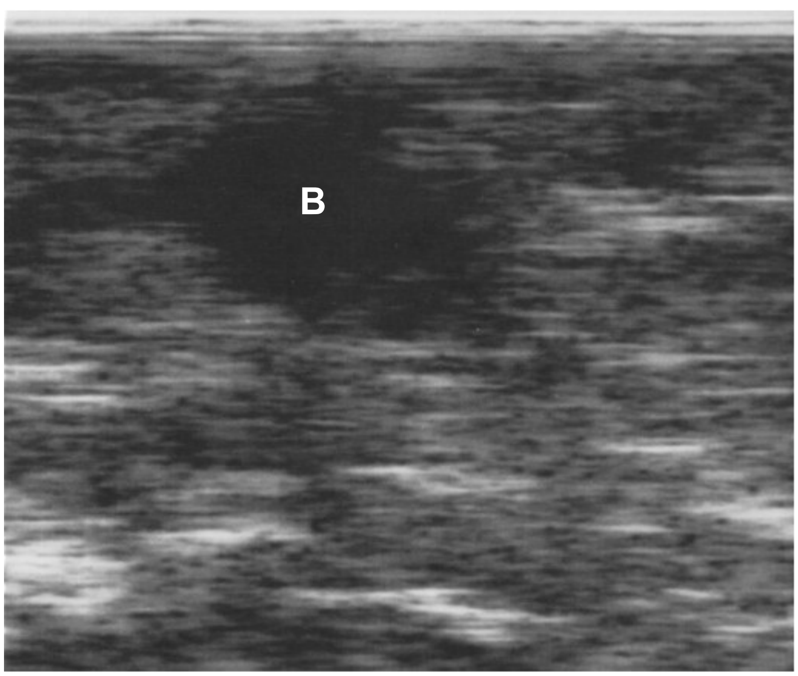

Figure 2. Longitudinal ultrasound scan of the urinary bladder (B) of male water monitor lizard. (*Varanus marmoratus*) showing irregular hypoechoic walls with anechoic lumen.

Schildger *et al.*, 1994; Hernandez-Divers and Hernandez-Divers, 2001; Hochleitner and Hochleitner, 2004; Mader, 2006). At times, uric acid particles in suspension are visible, appearing as corpuscular echogenicities. In the present study, the urinary bladder appeared as an irregular elongated structure and seemed to have a bladder stalk.

There were no significant differences in the mean width of the urinary bladder between small and large varanids (Table 1). Mean length showed significant differences between sizes of varanids. Larger animals showed longer urinary bladder compared to smaller animals. The size of the urinary bladder varied depending on urine contents. Sometimes, it can be used as a reservoir to moderate dehydration (Davis and Denardo, 2007). Echo mean values between sizes showed no significant differences.

Testes

The testes were located cranial to the kidney, oval-shaped and have variable sizes depending on the animal's sexual activity, in agreement with the observation of Stahl (2007). The testes were found in the mid-dorsal abdomen, in agreement with the report of Redrobe (2006). The parenchyma of the testes appeared homogenously hypoechoic (Figure 3), similar to the description of Stahl (2007), but is contrary to the hyperechoic testes observed by Redrobe (2006). The testes may

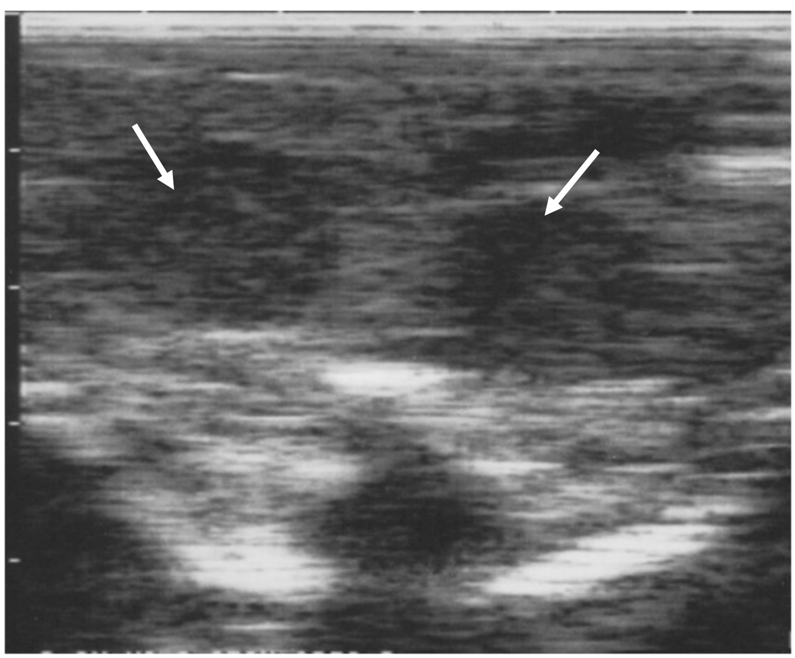

Figure 3. Transverse ultrasound scan of the testes (arrows) of water monitor lizard (*Varanus marmoratus*) showing rounded structures with hypoechoic parenchyma.

not always be documented in ultrasound; often they regress during non-breeding season and became more apparent towards the beginning of the breeding season (Mader, 2006). The color also changes seasonally, from grayish white to more yellow during the peak of reproductive phase (Auffenberg, 1988). Testes, which are slender and fusiform in shape, are difficult to image in snakes (Sainsbury and Gilic, 1991; Isaza *et al.,* 1993; Schildger *et al.*, 1994).

Statistical analysis showed no significant differences in the dimensions of right testes on all males with various sizes (Table 2). However, there were significant differences on the size of left testes between small and large varanids. Relatively, large animals have larger left testes in comparison to small ones. No significant differences in the echo mean values of the testes between left and right testes and across sizes were observed.

Table 2. Mean±SD dimensions and echo mean values of the testes in *Varanus marmoratus* (Weigmann, 1834).

Organ/ Animal	Length (cm)	Width (cm)	Circumference (cm)	Echo mean value
Left testis				
Small	$2.99{\pm}0.65^{a}$	$1.89{\pm}0.41^{a}$	$7.76{\pm}1.67^{a}$	$92.27{\pm}23.76^{a}$
Large	$3.22{\pm}0.22^{b}$	$2.08{\pm}0.54^{b}$	$9.07{\pm}1.66^{b}$	$81.62{\pm}28.44^{a}$
Average	3.11±0.47	1.98±0.46	8.41±1.69	86.94±24.92
Right testis				
Small	$3.28{\pm}0.75^{a}$	$2.06{\pm}0.48^{a}$	$8.56{\pm}1.63^{a}$	$80.88{\pm}8.32^{a}$
Large	$3.19{\pm}0.82^{a}$	$2.33{\pm}0.50^{a}$	$8.72{\pm}1.84^{a}$	$71.54{\pm}16.92^{a}$
Average	3.23±0.73	2.19±0.49	8.64±1.61	76.21±13.31

Means in the same column from the same organ with different superscripts are different ($P<0.05$).

Hemipenes

The hemipenes of *V. marmoratus* appeared as an elongated structure, narrowing from cranial to caudal, with hypoechoic walls and anechoic to hypoechoic middle (Figure 4). This is similar to the findings of Gnudi *et al.*, (2009) in snakes. They described the hemipenes as funnel-shaped with an echogenic thin wall and hypo to anechoic contents.

Male lizards have paired structures called hemipenes. It is a sac-like organ and lacks erectile tissues. These hemepenes when at rest lie invaginated within the hemipenal pockets distal to the vent. It is everted and lies lateral to the anus when engorged. The retractor muscle attached to the deep end of the hemipenal pocket stretches and pulls it back when the engorgement resolves. The hemepenes are used alternately, thus one may be slightly longer than the other (Paré, 2006) but they are not involved in urination (Barten, 2006).

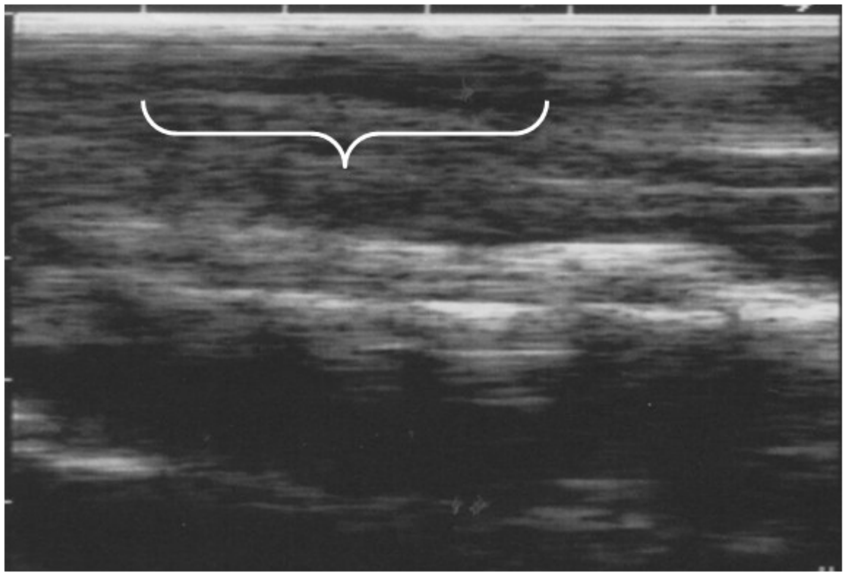

Figure 4. Longitudinal ultrasound scan of the hemipenis (bracket) of male water monitor lizard (*Varanus marmoratus*), showing hypoechoic walls with anechoic lumen.

The dimensions of the left and right hemipenes are shown in Table 3. There were significant differences between the hemipenes across sizes. Large animals have longer hemipenes than small varanids. Echo mean values showed no significant differences between sizes.

Table 3. Mean±S.D. dimensions and echo mean values of the hemipenes of *Varanus marmoratus* (Weigmann, 1834).

Organ / Animal	Length (cm)	Width (cm)	Echo mean value
Left hemipenis			
Small	5.76±1.00[a]	0.56±0.31[a]	58.55±3.65[a]
Large	6.16±0.55[b]	0.64±0.15[a]	46.67±3.49[a]
Average	5.96±0.51	0.60±0.17	52.61±7.64
Right hemipenis			
Small	5.84±1.15[a]	0.58±0.37[a]	55.96±6.56[a]
Large	6.06±0.44[b]	0.62±0.14[a]	51.02±10.60[a]
Average	6.00±0.45	0.60±0.18	53.49±8.15

Means in the same column from the same organ with different superscripts are different ($P<0.05$).

The results of the present study can serve as baseline information for evaluating diseases and disorders of the urogenital organs of male water monitor lizards.

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